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Serum Erythropoietin Levels in Children With Leukemia

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Objective. Our aim was to test the hypothesis that, in leukemic children, serum erythropoietin (EPO) levels vary inversely with hemoglobin.

Design. Twenty-four children (15 males, nine females) with an age range of 1–16 years (mean, 7.7 years) diagnosed with acute leukemia (22 acute lymphocytic, two acute myeloid) were studied over 4 months. Serum EPO and hemoglobin were measured simultaneously at multiple time points in the course of their disease, and a multiple regression analysis was performed to describe the EPO-hemoglobin relationship.

Results. In a model adjusted for individual subject, there was a significant correlation between hemoglobin and logEPO in these leukemic children ($r = -0.55$, $P < .01$, $n = 100$). When measurements at hemoglobins less than 10.0 were analyzed the correlation increased significantly ($r = -0.88$, $P < .01$, $n = 21$). However, approximately 20% of the observations fell into one of two groups: an inappropriately low EPO for hemoglobin or an inappropriately elevated EPO for hemoglobin. The clinical characteristics of the children at each of these determinations were not different in any manner from the determinations which fell within the 95% confidence intervals for predicted mean EPO value; each of the outlying points came from a patient who at other times had an appropriate EPO for hemoglobin.

Conclusions. There existed a significant inverse relationship between hemoglobin and EPO, suggesting that the feedback mechanism for EPO is intact. Reasons for inappropriately high or low EPO, for level of hemoglobin, are not clear and may be reflective of other aspects of bone marrow or EPO metabolism. **Med. Pediatr. Oncol. 28:259–267.**

Key words: erythropoietin; leukemia; pediatric; anemia

INTRODUCTION

Human serum erythropoietin (EPO) is a hematopoietic growth factor produced and secreted by the peritubular interstitial cells of the kidney which acts on the erythroid progenitor cells of the bone marrow to stimulate proliferation, differentiation, and maturation of the erythroid cell line. EPO production is regulated by the relative oxygen tension of the cells involved in its synthesis [1,2]. Therefore, hypoxia, anemia, and cardiovascular insufficiency enhance its production [3]. Other factors may influence the production of EPO since levels are relatively higher for the degree of anemia in patients with erythroid hypoplasia; specific therapy such as iron or vitamin B_{12} may decrease EPO levels prior to changes in hemoglobin, and EPO levels can be affected by the administration of cytostatic agents [4]. In healthy, nonanemic adults and children over the age of 2 months, standard values of EPO have been established and range from 1.0 to 21.9 mU/mL in males and 1.1 to 20.5 mU/mL in females [5]. In these nonanemic populations, there is no clear relationship between hemoglobin and EPO levels [6].

Erythropoietin response to anemia appears to be related to the cause of anemia. Levels are highest in patients with primary erythroid hypoplasia and are elevated in patients with either iron-deficiency anemia or hemolytic anemia. Previous studies have shown an inverse relationship between EPO level and degree of anemia with a great degree of interindividual variation [7,8]. On the other hand, the EPO response is blunted in patients with anemia of prematurity [9], end-stage renal disease [8,10], the anemia of chronic disease [11], and sickle cell anemia [12].

Anemia is a common finding in patients with malignancy, and its etiology is multifactorial. In adults with malignancies, the EPO response to anemia does not consistently demonstrate the normal inverse relationship [13–15]. Few studies have been done in children with

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malignancy. Kalmanti and investigators demonstrated relatively normal mean EPO levels in children with lymphomas and solid tumors [16]. While the expected inverse relationship between anemia and EPO level existed in the children with lymphomas, it was not present in those with solid tumors. Children with solid tumors had EPO levels which where lower than expected for the degree of anemia [16]. The EPO levels and response to anemia in children with leukemia at various stages of the disease has been investigated previously [17]. In those children, the expected inverse relationship between degree of anemia and EPO level was present at diagnosis. However, EPO levels increased following the administration of high-dose methotrexate (MTX) without a concomitant alteration in hemoglobin. There was, however, a consistent increase in EPO following treatment with cytosine arabinoside (Ara-C) associated with the decline in hemoglobin levels. Additionally, EPO levels were slightly elevated without associated anemia in some but not all patients during oral maintenance therapy. The reasons for these unexpected findings were not clear.

Because of the paucity of information concerning levels of EPO in children with leukemia, we initiated this study to determine the levels of EPO relative to the degree of anemia in children with acute leukemia undergoing treatment at The Children's Memorial Hospital in Chicago. Knowledge about the nature of the EPO response in these children may add to the understanding of the mechanisms of anemia in children with leukemia and may prove useful in devising strategies employing exogenous hematopoietic growth factors in the supportive care of children with leukemia. The degree of endogenous EPO response may effect the response to exogenous EPO and, specifically, may be related to the dose of exogenous EPO needed to produce a clinically significant response.

MATERIALS AND METHODS

Between November 1, 1990 and March 30, 1991, 24 children with leukemia were studied at the Children's Memorial Hospital, Chicago during routine clinic visits or hospitalizations for chemotherapy, fever and neutropenia, or other intercurrent illnesses. The experimental protocol was approved by the Institutional Review Board of the Children's Memorial Hospital, and written, informed consent was obtained from the patient's parents and/or the patient prior to study. Eligibility criteria included a diagnosis of acute leukemia, available venous access, and written informed consent. There were no other specific selection criteria for entry into the study; subjects represent a convenience sample of patients presenting to the clinic or hospital during the time interval. Blood samples were drawn at the time of venous access for treatment or diagnostic purposes.

The initial laboratory studies performed included a complete blood count, reticulocyte count, serum iron, total iron-binding capacity, ferritin, and serum EPO level. Subsequent samples were obtained sequentially in patients for measurement of complete blood count, reticulocyte count, and EPO concentration. Iron status was assumed to remain stable throughout the course of the study. Serum creatinine was measured routinely during the course of therapy. When clinically indicated, patients were evaluated for the presence of cardiac or pulmonary disease, sepsis, and hypotension.

Phase of treatment, clinical status, and most recent red blood cell transfusion were documented at the time of each hemoglobin and EPO measurement. Since there were several different treatment regimens used in these patients, arbitrary definitions for phase of treatment were designated as follows: Initial was defined as prior to any therapy; induction, as therapy administered following diagnosis until remission was documented; consolidation, as any intensive, cyclic therapy after remission was achieved; maintenance, as continuous, nonintensive therapy. The numbers of patients studied at each of these points was determined by the numbers of patients available for study in each phase since patients were not all studied from the onset of treatment.

Serum for EPO determination was collected and frozen at −30°C in order to batch specimens for measurement. Immunoreactive EPO was measured by modification of an existing enzyme immunoassay (Amgen Diagnostics, Thousand Oaks, CA). Standards and unknowns were incubated in 96-well plates precoated with EPO monoclonal antibody. The wells were then incubated with a conjugate of anti-erythropoietin polyclonal antibody and horseradish peroxidase, and the reaction was stopped with the addition of acid. Absorbance was measured immediately at a wavelength of 450 nm with a reference wavelength of 610 nm in a microplate reader (MR 5000, Dynatech, Chantilly, VA). Standards and unknowns were run in duplicate, and the result was expressed as the average of the two. Each patient's samples obtained during the study were run in the same assay. The sensitivity of the assay was 2 mU/mL; inter- and intra-assay variation were 12 and 14%, respectively. The range of normal EPO concentrations in nonanemic children over age 2 years in our laboratory was 5–25 mU/mL, with a mean value of 11 mU/mL ($n = 74$).

Complete blood count was performed at the time of the blood draw by standard Coulter counter, and reticulocyte count was determined manually. For the purpose of our analysis, anemia was defined as a hemoglobin <10.0 g/dL.

Subsequent analysis showed that no patient was iron deficient. Serum creatinine was monitored as a routine part of therapy and was found to remain within normal limits in all patients during the study period. No patient

TABLE I. Erythropoietin (EPO) Measurements at Hemoglobin Levels < 10.0 g/dL*

Patient No.	Sex/age (yrs) diagnosis	Sample No.	Erythropoietin mU/mL (hemoglobin g/dL) at stage of treatment				Days since last RBC transfusion	Days since last EPO level	Events
			Initial	Induction	Consolidation	Maintenance			
1	Male/8	7				233 (7.4)	48	14	Fever/ neutropenia
	ALL-T	11				219 (8.1)	22	9	
2	Male/12	1			1,364 (7.3)		50	0	
	ALL-C								
3	Male/4	2			91 (8.4)		95	12	Sepsis
	ALL-C								
5	Female/5	1				762 (9.2)	60	0	Sepsis
	ALL-T								
6	Female/11	3			134 (8.9)		25	33	Fever/ gastroenteritis
	ALL-C	5			101 (9.6)		24	11	Fever/ neutropenia
7	Female/1	3			77 (9.9)		100	12	
	ALL-C	4			295 (8.5)		138	38	Fever/ pneumonia
14	Female/5	3			58 (9.2)		22	20	
	ALL-C	4			<u><2 (9.7)</u>		7	16	Fever
15	Male/13	1			1,252 (9.2)		7	0	
	AML	6			757 (9.7)		1	9	Fever/ neutropenia
16	Male/1	2			49 (9.3)		36	42	
	ALL-C	5			<u>6 (9.3)</u>		56	7	
17	Male/6								
	ALL-C	1	3,710 (6.1)				0	0	
18	Female/4	1	200 (9.3)				1	0	
	ALL-T								
19	Male/2	1		<u>4 (9.8)</u>			1	0	Fever/ gastroenteritis
	ALL-C	2			<u>20 (9.1)</u>		18	19	
		4				<u>13 (9.9)</u>	33	7	
20	Male/6	1		151 (9.9)			21	0	
	ALL-T								

*Underlined values indicate EPO values less than predicted for hemoglobin levels less than 10.0 g/dL (group A).
Abbreviations: ALL-C, ALL-T, Acute lymphocytic leukemia, common and T-cell; AMI, acute myeloid leukemia.

was clinically hypoxic at the time of any of the EPO measurements.

Multiple linear regression analysis was performed to produce a model with hemoglobin as the independent variable and log (base 10) EPO as the dependent variable. To adjust for within-subject variability and calculate 95% confidence intervals for the regression line, regression analysis using the ''mixed models procedure'' with the SAS statistical package was performed [18,19]. The confidence intervals shown represent the expected mean value of EPO for a given level of hemoglobin with 95% certainty for the sample population. In other words, the interval includes the true relation between hemoglobin and EPO with 95% probability. An F-test was used to determine statistical significance. Correlation coefficients were calculated after adjusting for repeated observations with a method previously described by Bland and Altman [20].

Data were analyzed for EPO response to level of hemoglobin and correlated to patient and treatment variables including phase of treatment, chemotherapy regimen, clinical status (e.g., presence of sepsis or hypoxia), and transfusion history.

RESULTS

Patient Characteristics

We studied 24 children with leukemia (15 male, nine female) whose ages ranged from 1 to 16 years (mean = 7.7 years, median 6.7 years). Twenty-two patients had acute lymphocytic leukemia (common n = 17, B-cell n = 1, T-cell n = 4), and two patients had acute myeloid leukemia. Over a period of 4 months, 100 EPO and hemoglobin levels were measured, each child having between one and 11 measurements, based on patient availability in the clinic during the study. These levels were obtained at initial diagnosis (n = 4), routine chemotherapy administration (n = 71), and during illness (n = 25). Measurements obtained at the time of routine chemotherapy administration were distributed throughout

TABLE II. Erythropoietin (EPO) Measurements at Hemoglobin Levels ⩾ 10 g/dL*

Patient No.	Sex/age (yrs) diagnosis	Sample No.	Erythropoietin mU/mL (hemoglobin g/dL) at stage of treatment				Days since last RBC transfusion	Days since last EPO level	Events
			Initial	Induction	Consolidation	Maintenance			
1	Male/8	1			130 (11.6)		14	0	
	ALL-T	2				99 (11.4)	21	8	
		3				8 (10.4)	4	18	Fever
		4				154 (10.5)	20	16	
		5				57 (10.0)	26	6	
		6				35 (11.6)	34	8	
		8				7 (12.5)	2	2	Fever/ neutropenia
		9				28 (11.4)	8	7	Fever
		10				6 (11.3)	13	4	
2	Male/12	2			17 (11.5)		9	10	
	ALL-C	3				22 (11.3)	55	46	
		4				32 (11.3)	62	7	
3	Male/4	1			191 (11.6)		83	0	
	ALL-C	3			20 (13.1)[a]		11	11	Fever
4	Female/4	1			<2 (12.2)		90	0	
	ALL-C	2			<2 (10.6)		92	2	
		3			10 (12.5)		104	13	
		4			<2 (12.4)		111	7	
		5			<2 (11.2)		133	20	
5	Female/5	2				2 (12.1)	9	9	
	ALL-T	3				47 (11.4)	40	31	
6	Female/11	1			14 (12.4)		29	0	
	ALL-C	2			265 (10.8)		35	6	
		4			18 (12.5)		13	13	
		6			5 (13.9)		0	1	Fever
		7			36 (10.9)		19	19	Fever
		8			8 (11.3)		22	3	
		9			10 (13.4)		28	6	
7	Female/1	1			22 (10.8)		87	0	
	ALL-C	2			9 (10.2)		89	2	
		5			<2 (10.9)		159	20	
		6			18 (11.3)		177	18	
		7				23 (10.0)	185	8	
8	Female/13	1				58 (12.0)	605	0	
	ALL-C	2				27 (12.4)	703	82	
9	Male/8	1				13 (13.0)	730	0	
	ALL-C	2				14 (14.1)[a]	785	56	
		3				24 (12.8)[a]	800	14	
		4				8 (12.8)	815	15	
10	Female/7	1			10 (11.4)		79	0	
	ALL-C	2			<2 (11.0)		122	43	
		3			<2 (11.6)		144	22	
11	Female/8 ALL-B	1				2 (13.5)	170	0	
12	Male/14	1			290 (13.2)		124	0	
	ALL-C	2			100 (12.9)		128	7	
		3			52 (12.4)		131	3	
		4			36 (13.3)		159	28	
		5				22 (14.4)[a]	183	24	
13	Male/8	1			31 (11.8)		163	0	
	ALL-C	2			12 (11.1)		216	26	
14	Female/5	1	274 (10.6)				1	0	Fever
	ALL-C	2		3 (11.9)			2	23	Fever
		5			13 (10.1)		26	19	
		6			17 (11.9)		33	9	

TABLE II. (Continued)

Patient No.	Sex/age (yrs) diagnosis	Sample No.	Erythropoietin mU/mL (hemoglobin g/dL) at stage of treatment				Days since last RBC transfusion	Days since last EPO level	Events
			Initial	Induction	Consolidation	Maintenance			
15	Male/13	2			520 (12.8)		7	28	Fever
	AML	3			23 (11.9)		13	6	Fever
		4			23 (10.9)		21	8	Fever
		5			258 (11.0)		52	30	
		7				68 (11.7)	13	12	
16	Male/1	1	891 (10.7)				1	0	
	ALL-C	3			23 (11.8)		42	6	
		4			6 (10.6)		49	7	
17	Male/6								
	ALL-C	2		72 (12.8)			1	3	
18	Female/4	2		3 (11.9)			4	8	
	ALL-T	3		6 (10.1)			9	5	
		4		<2 (15.5)			10	11	
		5		<2 (14.5)			12	2	
		6		156 (14.4)			17	5	
19	Male/2	3			7 (10.1)		27	9	
	ALL-C								
20	Male/6	2			12 (12.2)		10	15	
	ALL-T	3			60 (10.6)		23	13	
21	Male/5	1		130 (10.5)			12	0	
	ALL-C	2		94 (11.6)			1	4	
		3		14 (11.0)			11	10	
22	Male/14	1		16 (11.8)			2	0	Fever
	AML								
23	Male/16	1			22 (12.0)		20	0	
	ALL-C	2			29 (11.5)		22	2	
		3			92 (10.4)		24	2	
24	Male/3	1		2 (15.0)			1	0	Sepsis
	ALL-C								

*Underlined values indicate EPO levels higher than predicted for hemoglobin levels greater than 10.5 g/dL (group B).
Abbreviations: ALL-C, ALL-T, Acute lymphocytic leukemia, common and T-cell; AML, acute myeloid leukemia.
[a]Value within normal for laboratory (5–25 mU/mL).

the course of treatment encompassing a variety of treatment phases, including initial (n = 4), induction (n = 14), consolidation (n = 56), and maintenance (n = 26).

Hemoglobin measurements ranged from 6.1 to 15.5 g/dL (mean ± SD, 11.2 g/dL ± 1.7 g/dL), and the EPO levels ranged from undetectable (<2 mU/mL) to 3,710 mU/mL (mean ± SD, 142 mU/mL ± 425 mU/mL). EPO values, hemoglobin levels, and clinical characteristics for all measurements are summarized in Table I (measurements at hemoglobins <10 g/L) and Table II (measurements at hemoglobins ≥10 g/dL).

Relationship of EPO to Hemoglobin

There was a significant inverse linear relationship between hemoglobin and log EPO. (logEPO = 4.13 − 0.24 [hemoglobin], adjusted r = −0.55, $P < .01$). Figure 1 graphically represents this relationship and displays the 95% CI for the predicted mean value of logEPO for a given value of hemoglobin. In an analysis restricted to the 21 measurements with hemoglobin <10 g/dL, hemoglobin was even more highly correlated with logEPO (logEPO = 6.43 − 0.48 [hemoglobin]; adjusted r = 0.88, $P < .01$) (Fig. 2). In the six patients with moderate anemia (hemoglobin 6.1–8.5 g/dL), EPO levels exceeded 200 mU/mL (219–3,710) in all but one patient who had an EPO level of 91 mU/mL (hemoglobin 8.4 g/dL). It was evident that several observations of EPO levels deviated significantly from the 95% CI for the predicted mean EPO value and fell into one of two groupings.

Low serum EPO in the presence of anemia (group A). For five of the measurements at hemoglobins <10 g/dL (Figs. 1, 2, values marked with a solid circle) observed serum EPO was below the 95% CI, indicating a blunted EPO response. Their values are underlined in Table I. Three individuals accounted for these measurements, which were taken during induction (n = 1), consolidation (n = 3), and maintenance (n = 1). There was no observed difference in clinical or treatment characteristics between them and the remainder of the observations at all other time points. Meaningful statistical comparison of these points with the rest of the group was precluded by a small number of such observations. Fur-

thermore, each of these three patients had EPO levels that fell within the 95% CI at other measurements during the study.

High serum EPO in the presence of a normal hemoglobin (group B). For 23 of the measurements at hemoglobins >10.5 g/dL (Fig. 1, values marked with a solid triangle) observed serum EPO fell above the 95% CI. Their values are underlined in Table II. Four of these EPO measurements were within the normal range (5–25 mU/mL) for our laboratory. The remaining values occurred most commonly in patients during consolidation (n = 9), but also initial (n = 2), during induction (n = 3), or maintenance (n = 5). This distribution roughly corresponds to patient distribution among these treatment phases. Four of these measurements occurred in patients who had been transfused for severe anemia in the 24 to 36 hours preceding the measurement. The remaining measurements were performed during a variety of chemotherapeutic protocols and clinical events such as fever and neutropenia. Treatment characteristics as well as patient age and gender were not different from the characteristics of the remainder of the observations.

Transfusion Requirements

Transfusion requirements in the study group were examined during the 4 months following entry into the study. Overall, the 24 patients received 41 transfusions of packed red blood cells (PRBC) totaling 72 units. During this time, they also received 110 units of platelet concentrate and 12 units of fresh frozen plasma. Fifteen units of PRBC were administered within 4 weeks of initial diagnosis or diagnosis of relapse. Thus, 57 units of PRBC were given to 17 patients with a median of 3 units per patient.

In group A, those children with relatively low EPO response, two patients received one unit of PRBC each after induction. Fourteen patients in group B, who had at least one measured EPO level which exceeded the expected response to the degree of anemia, received a total of 52 units of PRBC in the studied time interval. Two of four patients who had an EPO response appropriate to the level of hemoglobin received 3 units of PRBC.

DISCUSSION AND CONCLUSIONS

Our study describes the relationship between erythropoietin and hemoglobin in a small group of pediatric patients with leukemia. We have demonstrated that there is a significant inverse exponential relationship between hemoglobin and erythropoietin in this group and thus conclude that their ''feedback'' mechanism for erythropoietin production appears to be essentially intact. Our findings are similar to previous studies of the hemoglobin-EPO relationship in patients with iron-deficiency anemia, which have calculated correlations from −0.71 to −0.90 [7,13,21,22]. Bray and others, in an investigation of iron-deficient children with hemoglobin levels under 10g/dL, found that the regression between logEPO and hemoglobin yielded a slope of −0.55 and a correlation coefficient of −0.90 [7], which is remarkably similar to our slope of −0.48 and correlation of −0.88 in anemic patients. Although it was not possible to make a statistical comparison of these regression lines, the similar values of the slope and correlation provide evidence that the relationship between EPO and hemoglobin in these two disease states is quantitatively and qualititatively very similar.

Our findings are consistent with the study by Hellebostad et al., which showed that there was a strong inverse correlation between logEPO and hemoglobin in children with leukemia at time of diagnosis [17]. Similar to that study, several (seven of 12) of our study patients exposed to high-dose MTX had EPO levels which were higher than anticipated; small sample size precluded meaningful statistical analysis. Because of the wide variety of chemotherapeutic agents used in our study we were not able to isolate an effect of a specific chemotherapeutic agent.

Our findings are in contrast to studies which show a ''blunted response'' in adult patients with anemia and malignancy [13–15]. Whether this discrepancy is due to large individual variation, a more intact response in children, differences in disease process and treatment, overall clinical condition, or etiology of anemia is not clear. The patients in the current study were generally early in disease or in remission; anemia which occurred after initial presentation was most commonly secondary to chemotherapy rather than to malignancy. Furthermore, drugs such as cisplatinum, known to suppress EPO responses through renal toxicity, were not used in these children.

Notably, our data indicate that, at times, EPO levels are not well predicted by hemoglobin concentration and serum EPO is inappropriately high or low for a given level of hemoglobin. This observation is similar to such findings in several adult studies [13,14,23]. Reasons for this variation are not immediately apparent in our study. There was not an apparent correlation found between EPO levels and clinical illness (fever and neutropenia or other infection), type of chemotherapeutic agent, number of blood transfusions, age, gender of patient, or type of leukemia. However, owing to the fact that the study group had heterogeneous clinical and treatment characteristics, meaningful statistical comparisons cannot be made.

Relatively few of our observations (5%) demonstrated EPO levels which were lower than predicted for given level of hemoglobin (group A). Three of these observations occurred in one child, who despite a subnormal EPO response, was asymptomatic and maintained a hemoglobin level above 9.0 g/dL without transfusion, suggesting a resetting of the ''normal, nonanemic'' hemo-

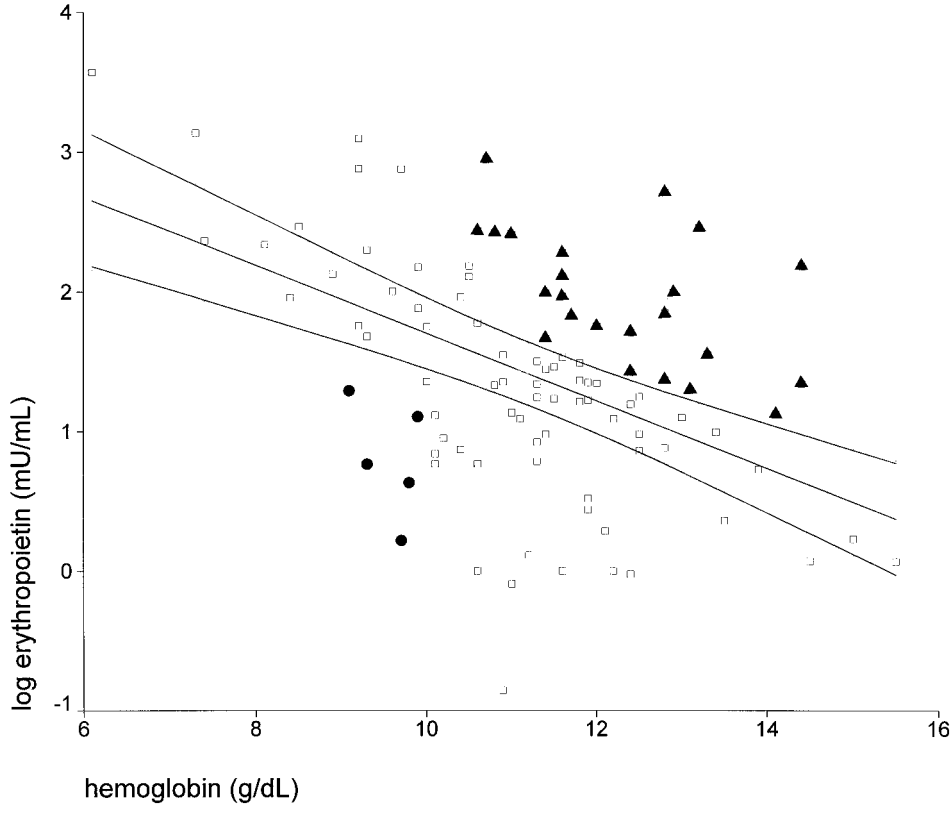

Fig. 1. The relationship between hemoglobin and serum erythropoietin (EPO) in a group of 24 leukemic children (n = 100 measurements). Regression equation: log EPO = 4.13 − 0.24 (hemoglobin); adjusted r = −0.55. Measurements with hemoglobin <10g/dL and EPO less than the lower limit of the 95% confidence interval for predicted value are marked with a solid circle. Measurements with hemoglobins >10.5 g/dL and EPO greater than the upper limit of the 95% confidence interval for the predicted value are marked with a solid triangle. All other measurements are marked with an open square.

globin. Overall, there are no obvious explanations for these observations with subnormal EPO responses.

Etiologies of an inappropriately low EPO level for concentration of hemoglobin fit into two broad categories: 1) decreased production of EPO and 2) increased catabolism of EPO. The first etiology is demonstrated by the anemia found in chronic renal insufficiency [24], for which our patients had no clinical or laboratory evidence. Decreased EPO could also be the result of an alteration of the regulatory protein(s) responsible for controlling its production. The protein which triggers the production of EPO is thought to sense hypoxia and is theorized to be a heme protein [25]. We speculate that this protein may be altered as a result of chemotherapy, rendering it unable to adequately ''sense'' hypoxia secondary to anemia.

Increased catabolism of EPO may reflect when red cell precursors in the bone marrow have a greater requirement for, and thus metabolize EPO more quickly, resulting in an inappropriately low serum EPO concentration. During marrow recovery, when erythroid cell lines are rapidly proliferating, there theoretically may be increased catabolism of EPO which is not balanced with an increased renal production. Marrow recovery occurs frequently in patients receiving chemotherapy. The paucity of patients demonstrating relatively low EPO responses suggests that increased catabolism in this manner is probably not occurring regularly in these patients. Thus, the rare blunted EPO response remains largely unexplained.

Similarly, reasons for an inappropriately high level of serum EPO for hemoglobin were not clear. In a few of the measurements the patient had been anemic and received a red blood cell transfusion within 24–48 hours prior to the EPO measurement. A high serum EPO could be reflective of a low hemoglobin in the recent past with the elevated EPO level resulting from the expected half-life of EPO degradation. Two of the observations occurred coincident with clinical sepsis and could have resulted from tissue hypoxia. Eight of these observations were seen in patients concurrently receiving MTX and/or Ara C, drugs reported to be associated with unexpectedly high EPO levels [17]. Ara C may produce erythroid hypoplasia which may lead to relatively high levels of EPO as are seen in red cell aplasia (Blackfan-Diamond anemia or transient erythroblastopenia of childhood) because of decreased utilization by erythroid progenitor cells [7]. MTX is known to cause intracellular folate deficiency and macrocytic anemia and may have an effect on EPO

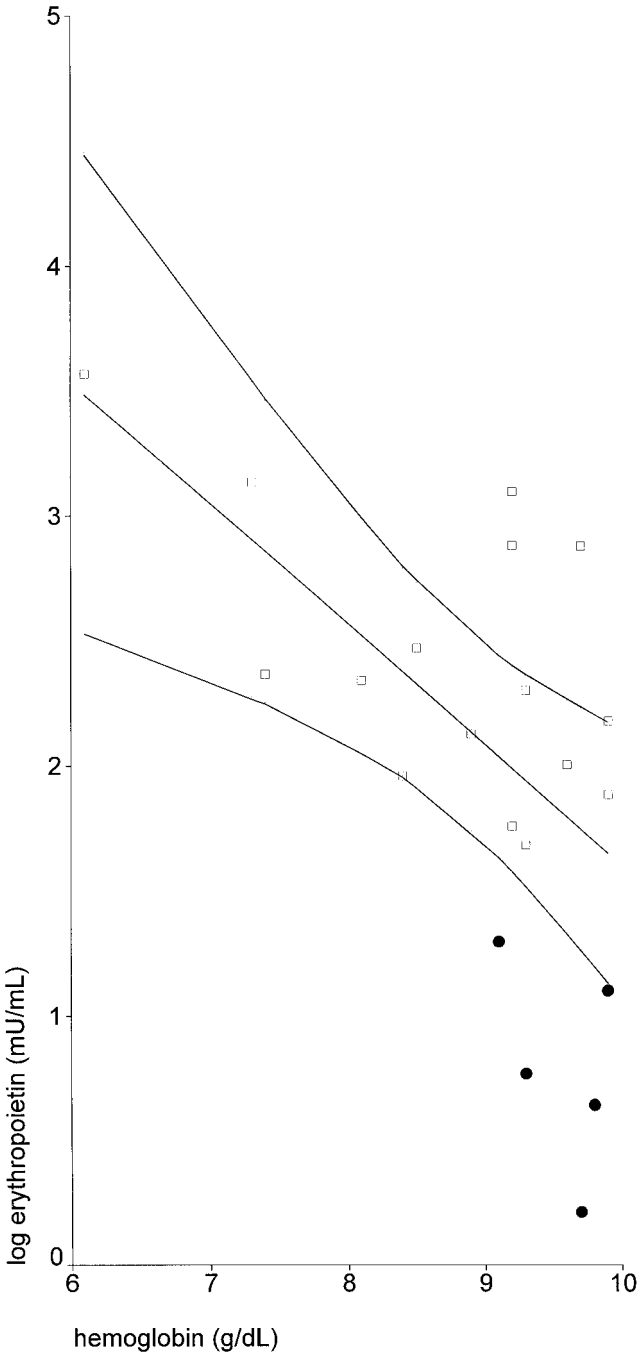

Fig. 2. The relationship between hemoglobin and serum erythropoietin (EPO) in a group of 13 leukemic children with hemoglobins <10 g/dL. Regression equation: log EPO = 6.43 − 0.48 (hemoglobin); adjusted r = −0.88. Measurements with EPO less than the lower limt of the 95% confidence interval for predicted value are marked with a solid circle. All other measurements are marked with an open square.

production which is not directly correlated to the degree of anemia. Parenthetically, in patients with HIV infection, it has been observed that EPO levels are low for hemoglobin but that when treated with zidovudine (AZT), another antimetabolite which consistently causes macrocytosis, EPO levels increase significantly [21]. The exact mechanism for this increase in this population is currently not understood but may be similar to that in our study population. An effect of a chemotherapeutic agent on the oxygen-sensing protein or at another point in the feedback mechanism has been postulated to influence EPO levels [14]. Further pharmacokinetic studies are needed to elucidate these mechanisms and to further understand the physiology of erythropoietin and its variation in disease states and under various therapeutic regimens.

The fact that the same patient or similar patients had measurements which were high, low, and normal for degree of anemia is not easily explainable. Patients on similar treatment plans had measurements which fell into all three categories, thus, basing an explanation solely on effect of chemotherapy as unwarranted. In addition, all patients who had ''inappropriately'' high or low values of EPO had values in the predicted range at other times in the course of their illness, and thus an explanation for aberrant values solely on the basis of individual variation is not warranted either. Single measurements are static and may not accurately reflect the dynamic relationships of EPO production and catabolism of EPO. Overall, it may be concluded that determinants of EPO response in our patient population are likely to be multifactorial.

To define an EPO level as ''appropriate'' to the level of hemoglobin requires that the disease process be taken into consideration, since EPO requirements and metabolism may vary greatly. Despite these findings, the fact that there is a significant inverse relationship between hemoglobin and serum EPO must be considered if treatment of anemias in leukemic children with r-HuEPO is entertained.

Response to r-HuEPO therapy has been well correlated with endogenous levels in other anemic patients with malignancy [26,27], and moderate doses are required in patients with endogenous EPO levels between 100 and 500 mU/mL. Thus, future studies in pediatric patients with leukemia using r-HuEPO should take into account endogenous levels of EPO. Patients who are anemic and have lower levels of EPO for level of hemoglobin may benefit from administration of exogenous EPO as a physiologic replacement. In patients with endogenous levels of EPO that are normal or high relative to hemoglobin level, then r-HuEPO may be of some benefit as a pharmacologic agent, increasing EPO levels to supraphysiologic levels by increasing dosage level. As the majority of patients in our study had ''normal'' or higher-than-expected EPO responses, one would anticipate that such patients would require higher doses of exogenous EPO. Since the majority of these patients will have received transfusions at presentation or during initial treatment at a time when exogenous EPO would not have sufficient time to be effective, the risk of infection from transfusion would be only reduced partially. Most

of these patients, however, had minimal transfusion requirements and there may be a subset of patients not represented here where reduction in red cell substitution might create a more substantial benefit.

Response to exogenous EPO in leukemic children is unknown. Likewise, toxicity and long-term effects of such therapy in this population and in conjunction potentially with administration of other growth factors (e.g. G-CSF (granulocyte-colony-stimulating factor), granulocyte macrophage–colony-stimulating factor) have not been studied. Exogenous administration of EPO has been studied in patients after bone marrow transplantation in which high-dose exogenous EPO (150 1U/kg/day) did not accelerate recovery of erythropoiesis following autologous transplant [28].

Therefore, recommendations for administration of exogenous EPO should consider the degree of anemia and its physical consequences as well as cost effectiveness compared to intermittent red blood cell transfusions and the relative risk of transfusions in patients who may still require exposure to platelet products and occasional red cell units. Prospective studies on the administration of r-HuEPO must evaluate all of these factors as well as the patients' endogenous EPO production to provide accurate information concerning utility of this product in varying clinical settings and treatment protocols.

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